Background Information

In humans, DNA is packaged into 23 pairs of chromosomes. Although most of this DNA is identical between individuals, small sequence differences, or "polymorphisms", occur at specific locations throughout the genome. These polymorphisms include single base pair changes and repetitive DNA elements. Analyzing several polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another.

The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are used to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. First, a suitable sample must be found. Forensic scientists use great care collecting evidence from crime scenes so that the DNA will not be damaged. After DNA is extracted from these samples, forensic scientists analyze the sample to create a DNA fingerprint. The DNA fingerprint from a crime scene can then be compared to the DNA fingerprints of different suspects. A match provides strong evidence that the suspect was present at the crime scene.

Early fingerprinting analysis involved treating the isolated DNA with special enzymes called restriction endonucleases, which act like molecular scissors to cut DNA at specific sites. Based on the distances between recognition sites, digestion of DNA by a restriction enzyme will produce DNA fragments of varying lengths. After electrophoresis of the digested sample, the DNA is transferred to a nylon membrane during a process known as Southern blotting. Sequence-specific DNA probes are used to visualize the membrane-bound DNA. If the DNA is not digested by the restriction enzyme, the probes will only hybridize to a single DNA segment. If a restriction site occurs within this sequence, the probe will hybridize with multiple bands of DNA. This technique, called Restriction Fragment Length Polymorphism (RFLP) analysis, was first used in a criminal investigation in the mid-1980s (summarized in Figure 1).

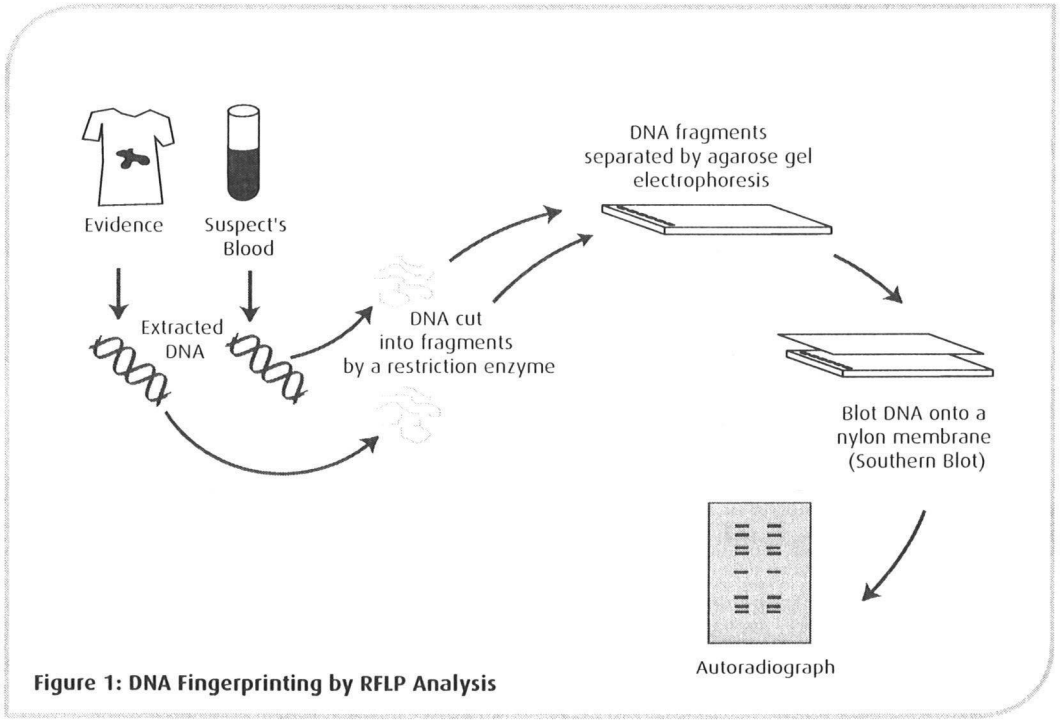


Figure 1: DNA Fingerprinting by RFLP Analysis

Background Information

Although RFLP analysis is very precise, it is time-consuming and requires large amounts of DNA. To address these problems, forensic scientists use the polymerase chain reaction (PCR) to produce DNA fingerprints. PCR allows researchers to quickly create many copies of a specific region of DNA *in vitro*. This technique requires 500-fold less DNA than traditional RFLP analysis and it can be performed in an afternoon.

To perform PCR, purified double-stranded DNA is mixed with primers (short synthetic DNA molecules that target DNA for amplification), a thermostable DNA polymerase (*Taq*) and nucleotides. The mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (Figure 2). To produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to rapidly heat and cool the samples.

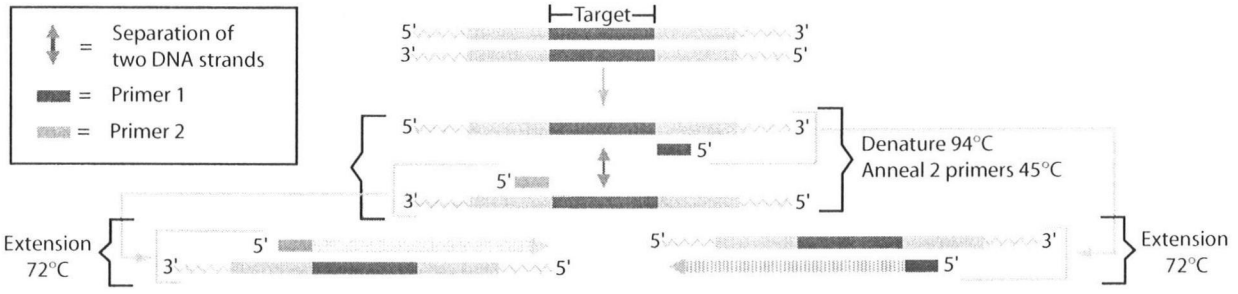


Figure 2: Amplification of DNA by PCR

After the crime scene sample is analyzed using electrophoresis, it is compared to DNA fingerprints from the suspects or those stored in CODIS (COmbined DNA Index System), a database of DNA fingerprints from convicted offenders, other crime scenes, and missing persons. Each DNA fingerprint analyzes thirteen separate loci, making the odds of an exact match less than one in a trillion. This evidence is then used in court to link a suspect to a crime scene.

In this forensic DNA fingerprinting experiment, students will perform RFLP analysis to link a suspect to a crime scene. First, a biological sample was collected at the crime scene. Next, samples were collected from two suspects. The DNA was extracted and digested using two different restriction enzymes before being separated using agarose gel electrophoresis. Each pair of restriction digests will create an individual's unique fingerprint. The goal is to analyze the DNA fingerprint patterns after agarose gel electrophoresis and to decide if Suspect 1 or Suspect 2 was at the crime scene. The DNA fragmentation patterns are simple enough to analyze directly in the agarose gel. In this experiment, the DNA fragments produced by restriction digest are represented by various dyes. The purple, blue, and orange bands represent DNA fragments of different sizes. By using brightly colored dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time.

Experiment Overview

EXPERIMENT OBJECTIVE:

This experiment explores the principles of DNA fingerprinting for the analysis of crime scene DNA. After performing agarose gel electrophoresis with colorful dyes, students will determine which suspect was at the crime scene.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

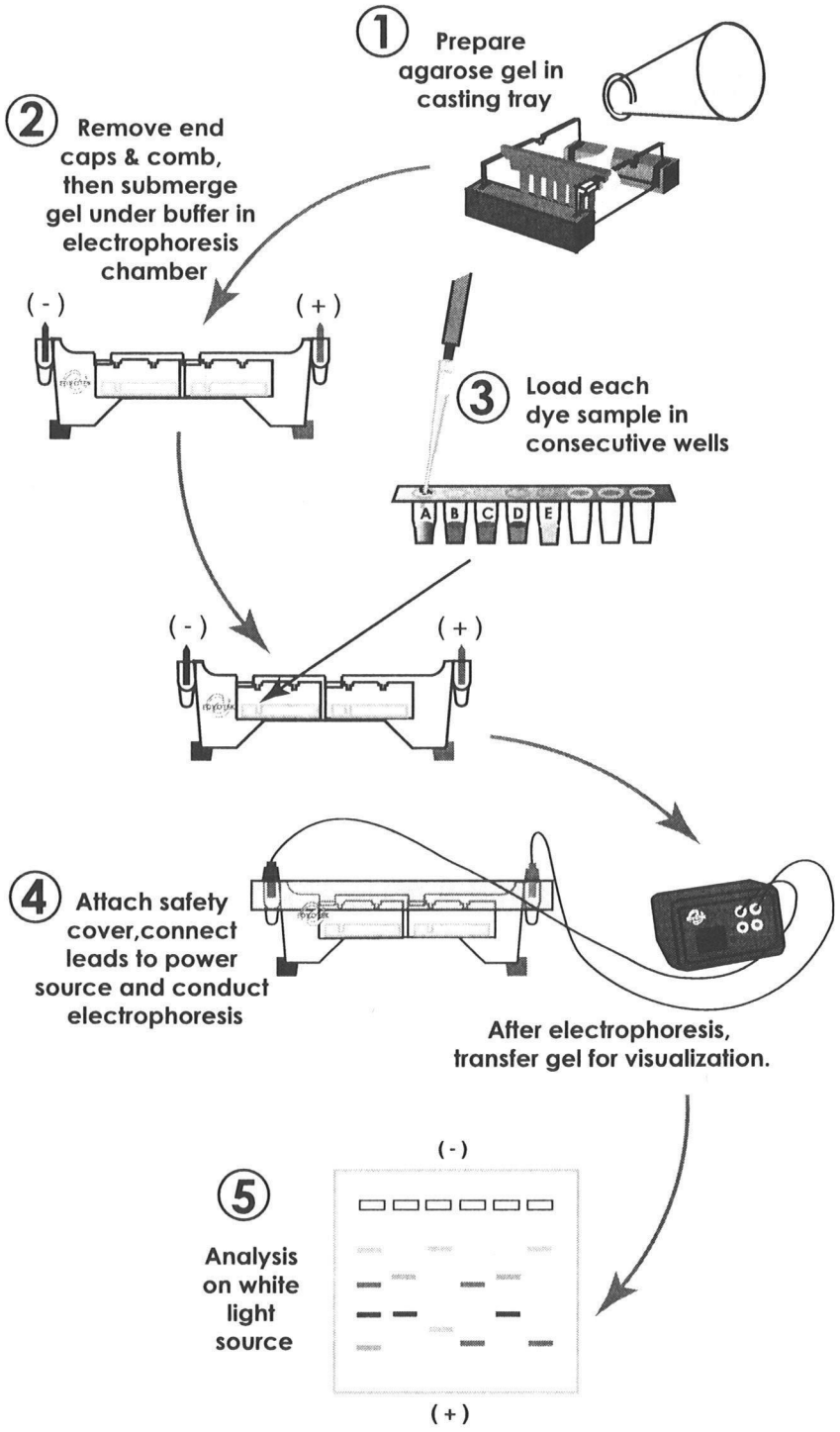
During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results - does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview



Gel pattern will vary depending upon the experiment.

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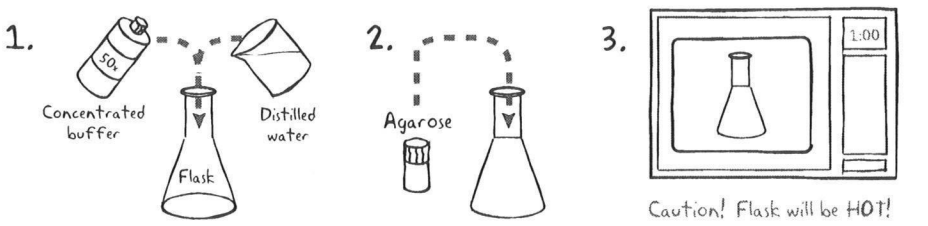
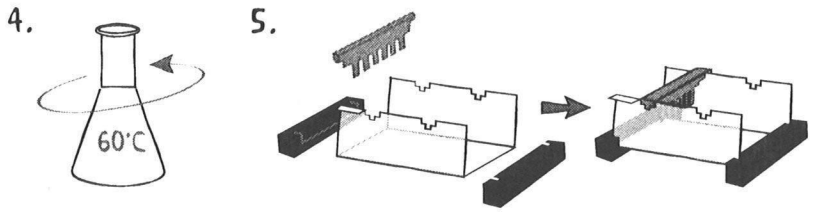
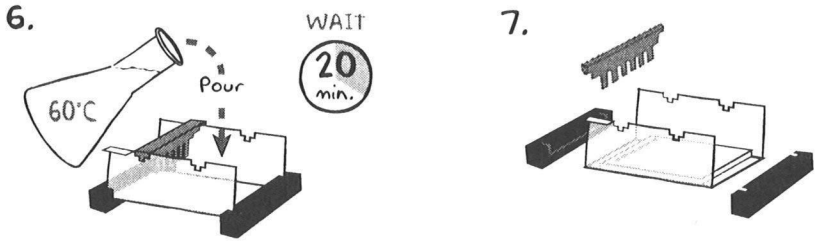
Agarose Gel Electrophoresis







IMPORTANT:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

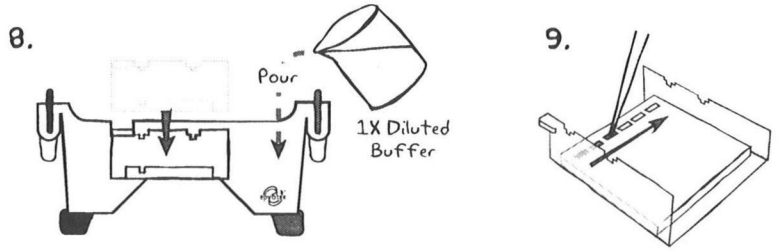
Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

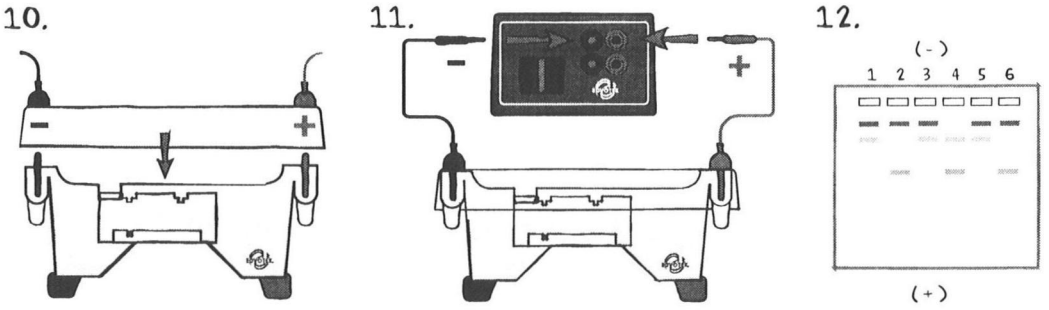
Agarose Gel Electrophoresis



Reminders:

If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38 µL) into the well in consecutive order. The identity of each sample is provided in Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the dye samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Crime Scene Simulated DNA Digest 1
2	Tube B	Crime Scene Simulated DNA Digest 2
3	Tube C	Suspect 1 DNA Digest 1
4	Tube D	Suspect 1 DNA Digest 2
5	Tube E	Suspect 2 DNA Digest 1
6	Tube F	Suspect 2 DNA Digest 2

Table B — 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+	Distilled Water
M6+	300 ml	6 ml		294 ml
M12	400 ml	8 ml		392 ml
M36	1000 ml	20 ml		980 ml

Table C — Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model M6+	Electrophoresis Model M12 & M36
Volts	Min. / Max.	Min. / Max.
150	15/20 min.	25 / 35 min.
125	20/30 min.	35 / 45 min.
75	35 / 45 min.	60 / 90 min.
50	50 / 80 min.	95 / 130 min.

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Study Questions

1. Why is it important to position the sample wells near the negative electrode?
2. What kind of evidence would you look for at a crime scene to obtain DNA?
3. How will you be able to tell who committed the crime?
4. Who is the suspect that committed the crime?
5. What determines that each person has a unique pattern within their DNA?
6. Can you think of a case when two people will have identical DNA patterns?